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p = 0.035) and higher overall survival (HR: 0.60, 95%CI: 0.36-0.99, p = 0.047) than those who received stem cells from a donor without the C-A-A haplotype. Additionally, the group with the C-A-A haplotype exhibited high disease-free survival (HR: 0.66, 95%CI: 0.41-1.06, p = 0.085), compared with the group without the haplotype C-A-A. The presence or absence of the C-A-A haplotype did not affect the incidence of acute and chronic GVHD, and non-relapse mortality. This is the first report described the effect of CTLA-4 haplotype, but not each SNP, on allogeneic HSCT. Since the presence of CTLA-4 haplotype C-A-A reduced the risk of relapse and improved survival after allogeneic HSCT, CTLA-4 haplotype may provide useful information for donor selection.

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EPIGENETIC CONTROL OF GVHD AND GVL USING THE HYPOMETHYLATING AGENT AZACITIDINE

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Allogeneic bone marrow transplantation (BMT) represents the most effective treatment for patients with high risk and relapsed hematologic malignancies because allogeneic donor T cells provide a graft-versus-leukemia (GvL) effect. However, the same cells can cause graft-versus-host disease (GvHD), one of the major complications. Regulatory T cells (Tregs) have been shown to suppress GvHD while preserving GvL, their use provides a promising strategy in the allogeneic transplant setting if three major obstacles will be overcome: 1) the low numbers of Tregs, 2) loss of suppressor activity following *ex vivo* expansion and 3) the lack of Treg-specific markers to purify *ex vivo* expanded Tregs. The FOXP3 transcription factor, which is exclusively expressed in Tregs, can convert effector T cells (Teff) into Tregs when ectopically overexpressed. The *Foxp3* locus is unmethylated in Tregs while highly methylated and silenced in all other T cells. The hypomethylating agent azacitidine (AzaC) can modulate this methylation status and induce stable FOXP3 expression (>7 days) in Teff. Furthermore, we have shown that these AzaC-induced FOXP3 + T cells are suppressive phenotype *in vitro*. Thus, we hypothesize that AzaC treatment of mice after allogeneic BMT will dramatically mitigate GvHD while preserving GvL via upregulation of *Foxp3* in alloreactive Teff.

In murine T cell depleted (TCD) BMT model (B6 → Balb/c) with delayed infusion of conventional T cells (Tconv) ($2X10^6$) at day 11 post BMT, followed by subcutaneous treatment of AzaC (2 mg/kg at days 15, 17, 19, and 21 post BMT), we found that AzaC dramatically suppressed GvHD caused by allogeneic donor T cells while maintaining donor engraftment of all lineages. The AzaC group had significantly higher FOXP3 + Tregs than in PBS control group and that these Tregs were derived from donor T cells, suggesting that the suppression of GvHD was mediated by AzaC-induced Tregs. We further tested whether AzaC treatment of mice transplanted with allogeneic T cells preserve GvL while mitigating GvHD. Using the same murine allogeneic BMT model, Click Beetle Red luciferase-expressing A20 leukemia cells (Balb/c derived; $1X10^4$) were injected with TCD BM and 10×10^6 Tconv and *in vivo* bioluminescence imaging was performed to assess tumor burden. We found that AzaC treatment mitigated GvHD without abrogating GvL or donor engraftment. Thus, the adminstration of hypomethylating agents like AzaC might be a promising strategy to treat GvHD.

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BLOCKADE OF TWO INDEPENDENT INNATE IMMUNITY PATHWAYS SYNERGISTICALLY PREVENT LETHAL GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease (GVHD) is a major complication following hematopoietic cell transplantation. Innate immunity plays a major role in the development of GVHD both by aiding allogeneic T cell responses and by damaging target organs directly. MyD88 is an adaptor protein for the majority of toll-like receptors. The complement system is a central component of the innate immune response. We studied the role of these two pathways in the development of GVHD using the C57BL/6 into BALB/c model. Lethally irradiated recipients of $MyD88^{-/-}$ T cells survived slightly longer than recipients of wild type cells (median survival time: 48 vs. 32 days, $P < 0.05$). Similarly, treatment with an anti-C5 antibody (clone BB5.1, 1 mg/dose, i.p., three times a week for 4 weeks), that blocks all three pathways of complement activation and prevents cell lysis, also moderately prolonged the survival of the mice with GVHD compared with the isotype control group (median survival time: 43 vs. 32 days). Treatment of $MyD88^{-/-}$ T cell recipients with anti-C5 antibody protected 80% of the animals from lethal GVHD at 100 days post transplantation, while only 20% in the $MyD88^{-/-}$ T cell recipients treated with isotype antibody group and 30% in the wildtype T cell recipients treated with anti-C5 antibody group survived (Table, $P < 0.01$, compared with other groups). All $MyD88^{-/-}$ T cell recipients treated with anti-C5 antibody developed GVHD. However, the disease was less severe in these animals than those in the recipients of a single pathway blockade as measured by body weight and other clinical signs of GVHD. The anti-C5 antibody did not seem to prevent GVHD through inhibiting T cell activation and expansion because it did not inhibit mixed lymphocyte reactions. Levels of multiple pro-inflammatory cytokines were lower in the $MyD88^{-/-}$ T cell recipients treated with anti-C5 antibody compared with those in the control groups. These data clearly demonstrate that, even though blockade of MyD88 or complement pathway alone may prevent GVHD, GVHD can be better controlled by blocking both MyD88 and complement pathways.

Blockade of two independent innate immunity pathways synergistically prevent lethal graft-versus-host disease

Groups	n	Survival at day +50	Survival at day +100
TCD BM alone	10	100%	100%
Wildtype + isotype	10	20%	10%
Wildtype + anti-C5	10	40%	30%
MyD88-/- + isotype	10	50%	20%
MyD88-/- + anti-C5	10	90%	80%

$P < 0.01$, MyD88-/- + anti-C5 vs. other groups. A representative experiment of three is shown.

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MYCOPHENOLATE PHARMACOKINETICS AND ASSOCIATION WITH RESPONSE TO ACUTE GRAFT VS HOST DISEASE (GVHD) TREATMENT

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Mycophenolate mofetil (MMF) is a common and effective prophylactic immune suppressant that promotes hematopoietic cell engraftment and prevents graft vs host disease (GVHD) after allogeneic hematopoietic stem cell transplant (HCT). However, there is limited data as to the effectiveness of MMF plus high dose corticosteroids for the treatment of acute GVHD and even less data regarding the pharmacokinetic disposition and exposure-response relationship of mycophenolate in individuals with GVHD. Mycophenolate pharmacokinetics were studied in a multi-center CTN randomized phase II trial evaluating the effectiveness of MMF as one if four novel agents added to steroids as initial treatment of acute GVHD. Thirty-two patients randomized to receive MMF 1 gm twice daily who underwent pharmacokinetic sampling in weeks 1 and 2 were studied. Median age was 41 ± 13.6 years. Twenty one (65.6%), 5 (15.6%), 6 (18.8%) patients had a complete response (CR), partial response (PR) or lesser response by day 28, respectively.

Twenty-five (78.1%), 2 (6.3%), 5 (15.6%) patients had a CR, PR, or other response by day 56 to treatment, respectively. Single mycophenolic acid (MPA) pharmacokinetic measurements during weeks 1 and 2 did not correlate with CR at either day 28 or 56 ($p > 0.07$). However, if the mean of weeks 1 and 2 total MPA troughs was >0.5 mcg/mL or unbound trough >0.015 mcg/mL, a significantly greater proportion achieved a CR + PR at day 28 and 56. A CR + PR at day 28 was observed in 19/19 (100%) of patients if the mean total trough was >0.5 mg/mL, but in only 7/13 (54%) if ≤0.5 mcg/mL ($p = 0.002$). Similarly, 15/15 (100%) individuals had a CR + PR at day 28 if their unbound MPA trough concentration was >0.015 mcg/mL while only 11/17 (65%) responded if trough was ≤0.015 mcg/mL ($p = 0.02$). There was no association with risk of infection by day 90, overall survival at day 180 post randomization and any pharmacokinetic measures. About one-half of subjects' therapy did not achieve the favorable MPA total and unbound trough targets. The current practice of MMF 1 gm twice daily dosing provides low plasma concentrations in many patients. Increased dosing at 3 gm/day may improve the efficacy of MMF as acute GVHD therapy.

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ASSOCIATION BETWEEN NUMBER OF PREDICTED MINOR HISTOCOMPATIBILITY ANTIGENS AND CLINICAL OUTCOME AFTER NON-MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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In fully HLA matched, allogeneic hematopoietic cell transplantation (HCT), minor histocompatibility antigens (miHAs) recognized by donor cytotoxic T cells are believed to be the main cause of graft versus host (GVH) disease and the graft-versus-tumor (GVT) effect. Differences in non-synonymous coding single nucleotide polymorphisms (nsSNPs) between donor and patient are the most common cause of miHAs. Approximately 30 miHAs, mostly pertaining to HLA-A*0201, have been identified so far, but considering the numerous different HLA-types in the human population and possible nsSNP differences between any two individuals it seems likely that many miHAs have yet to be identified. The objective of the current study was to predict the number of possible miHAs in 11 proteins using an *in silico* approach and assess the influence of the number of predicted miHAs on outcome after allogeneic HCT following non-myeloablative conditioning (NMC). miHA predictions were performed using the publicly available network based tool, netMHCpan, which can predict the binding of peptides to more than 1000 different HLA-A and B molecules. The study cohort consisted of 126 patients treated with allogeneic HCT following NMC (matched related donor, n = 70; matched unrelated donors, n = 56) for hematologic malignancies, and was genotyped for 53 nsSNPs, in 11 proteins known to contain miHAs. For each patient/donor pair, a predicted miHA in the GVH direction, was defined as a peptide with a nsSNP-variant unique to the patient and with a predicted binding to any of the HLA-A or –B molecules expressed by the patient and donor. Twenty-three nsSNPs within 6 proteins showed variation in the GVH-direction. Patients with more than 5 predicted miHAs had a significantly lower overall survival (40% vs 73%, $p = 0.002$, hazard ratio (HR) 2.6, $p = 0.003$) and treatment related mortality (38% vs 11%, $p = 0.019$, HR 3.2, $p = 0.016$) than patients with 5 or fewer predicted miHAs. No association between number of miHAs and any other clinical outcome parameter was observed. Collectively, our data suggest that six of the 11 proteins included in the current study could contain more miHAs, that have yet to be identified, and that the presence of multiple miHAs confers a higher risk of mortality after NMC conditioning HCT. Furthermore, our data suggest a possible role for *in silico* based miHA prediction, in both donor selection and in selecting candidate miHAs for further evaluation in *in vitro* and *in vivo* experiments.

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PR1-SPECIFIC T CELL RESPONSES IN THE FIRST MONTHS FOLLOWING T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION OCCUR IN BOTH MYELOID AND NON-MYELOID MALIGNANCIES BUT ARE ONLY ASSOCIATED WITH A GVL EFFECT IN MYELOID LEUKEMIAS

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Lymphopenia-driven homeostasis post-SCT allows expansions of donor T-cells against antigens (Ag). Leukemia-associated-Ag proteinase 3 (PR3) & elastase (ELA2) are self-Ag which induce low frequency autoreactive T-cells in normal controls. PR1, an HLA-A*0201 epitope shared by PR3 & ELA2, is expressed in normal neutrophils & overexpressed in myeloid (not lymphoid) leukemias. T-cells against PR1 have been linked to GVL. We looked for PR1-CD8 + T-cells in 28 patients (13 CML, 10 ALL, 5 solid tumors) at day +30-120 following T-cell depleted SCT, using PR1/HLA-A2 tetramers & IC-IFN-γ staining & correlated them with ELA2 & PR3 expression (using qRT-PCR) & GVL. Ten CML, 6 ALL & 3 solid tumor patients had PR1 responses post-SCT. PR3 & ELA2 expression was strongly associated with emergence of PR1-T-cells. Reduction in PR3 & ELA-2 expression coincided with disappearance of PR1-T-cells ($P < 0.001$). In-*vivo* anti-leukemia effect of the PR1 response was assessed in CML patients by BCR-ABL qRT-PCR: 9/10 patients with early PR1 responses were BCR-ABL negative at day +90 compared to 0/3 without ($P < 0.001$).This GVL association was restricted to CML: in ALL, using WT1 qRT-PCR to measure MRD, 2/5 patients with PR1 responses & 3/5 patients without were MRD+ on day 90 ($P = 0.36$). Since PR1 responses were not CML restricted and all patients had 100% donor myeloid chimerism by day 30, the recovering myeloid hematopoiesis from the donor is the likely antigenic source of PR3 & ELA2 driving the PR1 response. Based on this hypothesis we initiated a clinical trial in patients with relapsed AML to receive weekly vaccination with the WT1-126 peptide admixed with Montanide adjuvant plus GM-CSF following an immunotherapy approach of lymphodepletion by Fludarabine & infusion of autologous lymphocytes collected prior to chemotherapy. All 3 patients enrolled so far had detectable WT1-CD8 + T cells following vaccination. Interestingly, PR1-CD8 + T-cells were also detected in 3/3 evaluable patients during hematopoietic recovery, associated with increases in PR3 & ELA2 expression. These data suggest the induction of an 'auto-vaccination' process by the recovering marrow & that the lymphopenic milieu permits exaggerated weak autoimmune responses to normal self-Ag such as PR1. This immune response may result in GVL if the self-antigen is also expressed on the leukemia as in CML. Vaccination together with induction of T-cell homeostatic proliferation is likely to enhance the anti-leukemia response of SCT.

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CCR5$^+$ T CELLS MEDIATE ALLOIMMUNE RESPONSES IN HUMAN GRAFT VERSUS HOST DISEASE

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Graft-versus-host disease (GVHD), a common complication that is caused by donor T cells following allogeneic hematopoietic cell transplant (HCT). The function of CCR5 in GVHD has been primarily explored in murine models and the data suggested a complicated role of CCR5 in alloimmmune responses. We have identified unrelated MHC-matched HCT donors genetically lacking CCR5 (CCR5δ32) and correlated presence or absence of CCR5 with HCT outcomes. Patients were 18-50 years old, and they received cyclophosphamide and fractionated total body irradiation, unmanipulated marrow, and GVHD prophylaxis with cyclosporine and methotrexate. A total of 344 CML patients had CCR5 wild-type donors, 39 had CCR5δ32 heterozygous donors, and 8 had CCR5δ32 homozygous donors. Logistic regression model was used to assess the association between CCR5 genotype and acute GVHD, and Cox regression was used for chronic GVHD. Results in the Table suggested a trend of less GVHD among patients whose donors were CCR5δ32 homozygous compared to patients with wild-type or heterozygous donors.